Structure of the Tet Repressor and Tet Repressor–Operator Complexes in Solution from Electrooptical Measurements and Hydrodynamic Simulations

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ABSTRACT: The Tet repressor protein and *tet* operator DNA fragments and their complexes have been analyzed by electrooptical procedures. The protein shows a positive linear dichroism at 280 nm, a negative linear dichroism at 248 nm, and a strong permanent dipole moment of 3.5×10^{-27} C m, which is independent of the salt concentration within experimental accuracy. Its rotation time constant of 40 ns indicates an elongated structure, which is consistent with a prolate ellipsoid of 100 Å for the long axis and 40 Å for the short axis. The time constant can also be fitted by a cylinder of length 78 Å and diameter 37 Å, which is consistent with nuclease protection data reported on repressor–operator complexes, if the cylinder axis is aligned parallel to the DNA axis. Addition of tetracycline induces changes of the limit dichroism but very little change of the rotation time constant. The rotation time constants observed for the operator DNA fragments show some deviations from the values expected from their contour length; however, these deviations remain relatively small. Formation of repressor–operator complexes leads to some increase of the DNA rotation time constants. Simulations by bead models demonstrate that these time constants can be explained without any major change of the hydrodynamic dimension of the components. The data for the complexes are fitted by bead models with smooth bending of the DNA corresponding to a radius of curvature of 500 Å, but at the given accuracy, we cannot rule out that the DNA in the complex remains straight or is bent to a smaller radius of ~400 A. Thus, binding of the Tet repressor, which is a helix–turn–helix protein as judged from its sequence, to its operator seems to induce minor bending but does not induce strong bending of the DNA double helix.

The identification of helix–turn–helix motifs as the DNA binding domain of many proteins, which recognize specific nucleotide sequences, suggests the existence of some general rules for specific protein–nucleic acid interactions. Until now, the helix–turn–helix motif has been found by X-ray analysis for five proteins already; in addition, there are a large number of proteins with the same motif according to sequence analogy (Anderson et al., 1981; McKay & Steitz, 1981; Pabo & Lewis, 1982; Schevitz et al., 1985; Anderson et al., 1987; Pabo & Sauer, 1984). Since the structure analysis of complexes between helix–turn–helix proteins and their specific DNA is more difficult and so far has been given only in one case at a resolution of ~4 Å (Anderson et al., 1987), the general nature of the interactions remains to be established. It is possible, for example, that the structure of the double helix is changed upon binding. Recently, it has been demonstrated that one member of the class of proteins with a helix–turn–helix motif—the cAMP receptor—induces strong bending of the double helix, when the protein is bound to specific DNA fragments in the presence of the inducer cAMP (Kolb et al., 1983; Wu & Crothers, 1984; Porschke et al., 1984a; Weber & Steitz, 1984). The protein-induced DNA bending has been characterized quantitatively (Antosiewicz & Porschke, 1988) on the basis of the observed strong reduction of rotational relaxation time constants (Porschke et al, 1984a) obtained from electrooptical measurements (Fredericq & Houssier, 1973). In the present investigation, we use the same procedure to study another member of the class of specific DNA binding proteins with a helix–turn–helix motif. Although the structure of the Tn10-encoded Tet repressor (Hillen et al., 1984) is not yet known in molecular detail (Parge et al., 1984), sequence analogy strongly suggests that the DNA binding domain of this protein is folded in a helix–turn–helix motif (Isackson & Bertand, 1985). We have studied the structure of complexes formed by this protein with several specific DNA fragments using electrooptical methods. Because of a rather large anisotropy, the hydrodynamic dimensions of the protein could also be characterized by electrooptical measurements.

Materials and Methods

The Tet repressor protein was prepared as described by Oehmichen et al. (1984). The operator DNA fragments originating from RP1 [82 base pairs (bp)], Tn10 (76 bp), pSC101 (80 bp), and RA1 (74 bp) were constructed and prepared as described elsewhere (Tovar et al., unpublished results). The tandem operator sequences contained in these sequences have been characterized previously (Klock et al., 1985). The centers of the operators were at base pairs 32 and 64 for RP1, 15 and 45 for Tn10, 22 and 51 for pSC101, and 21 and 49 for RA1. The 185 bp Tn10 tet DNA with centers of operators at base pairs 79 and 113 was prepared as published (Hillen et al., 1982). The 120 bp DNA fragment containing the single *tet* operator O_1 (center at bp 79) from Tn10 was prepared as described (Wissmann et al., 1986). All the operator fragments had four single-stranded residues at each end resulting from preparation by *Eco*RI restriction nuclease. Blunt-ended HaeIII fragments with 43, 69, 84, 179, and 256 bp were prepared by standard procedures (Porschke, 1986). Both protein and DNA were dialyzed extensively

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against the buffers used for our measurements. Buffer "T" contained 5 mM Tris(hydroxymethyl)aminomethane, pH 8.0, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 mM 1.4 dithioerythritol; buffer "T–Na" contained 10 mM NaCl in addition to the components of buffer T; buffer "T–Mg" was composed as buffer T, but 0.1 mM EDTA was exchanged against 0.1 mM $MgCl_2$; finally, buffer "T–Mg–Na" contained 10 mM NaCl in addition to the components of buffer T–Mg.

The electric dichroism was measured at 20 °C by a pulse generator (Grünhagen, 1974) and an optical detection system (Porschke, 1980) as described. The samples were subjected to field pulses in the range from 2 to 70 kV/cm in a cell with 10-mm optical path length and a distance between the Pt electrodes of 5.9 mm. UV radiation damage was avoided by using an automatic shutter for short light pulses synchronized to the field pulses. Both UV transmission and electric field strength as a function of time were recorded by a Tektronix 7612D transient recorder. The stationary dichroism was evaluated by using graphic routines on a LSI11/23 (Porschke et al., 1984b), whereas time constants were evaluated after transmission of the data to the Univac 1108 of the Gesellschaft für wissenschaftliche Datenverarbeitung Göttingen by an efficient deconvolution routine (Porschke & Jung, 1985).

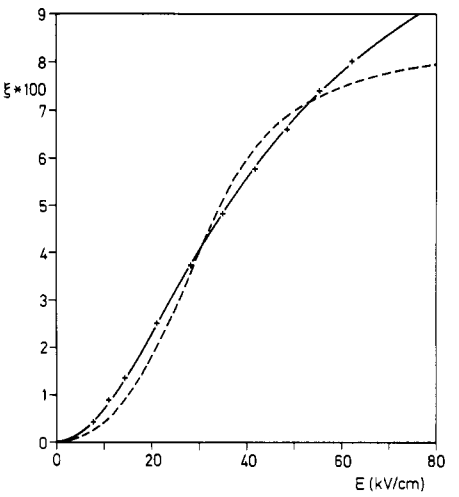

FIGURE 1: Reduced linear dichroism (ξ) as a function of the electric field strength (E) for the Tet repressor protein (buffer T, 20 °C, 280.4 nm). The continuous line shows a least-squares fit according to a permanent dipole orientation mechanism with a limit dichroism $\xi_\infty = 0.15$ and a permanent dipole moment of 3.5×10^{-27} C m. The dashed line shows a least-squares fit according to an induced dipole model.

RESULTS

Dipole Moment and Approximate Dimensions of the Protein. When solutions of the Tet repressor protein are exposed to electric field pulses, the absorbance of light at 280.4 nm, which is polarized parallel to the field vector, increases due to field-induced alignment of the molecules. This assignment is supported by the absence of absorbance changes at the magic angle orientation of polarized light and by reversal of the amplitude together with a reduction by a factor of 2 for perpendicular orientation of light and field vectors. The extent of molecular alignment is reflected by the linear dichroism:

$$\xi = \frac{\Delta A_\parallel - \Delta A_\perp}{A}$$

where $\Delta A_\parallel$ ($\Delta A_\perp$) is the absorbance change for light polarized parallel (perpendicular) to the field vector and A is the isotropic absorbance (Fredericq & Houssier, 1973). The stationary value of the dichroism at a given field strength (E) increases with E as shown in Figure 1. The experimental data can be fitted at high accuracy by an orientation mechanism on the basis of a permanent dipole moment, whereas an induced dipole mechanism is not consistent with the data (cf. Figure 1). The permanent dipole moment, $\mu_p = 3.5 \times 10^{-27}$ C m, obtained from a least-squares fit is considerable and corresponds to 1050 D. From the same least-squares fit, we obtain a limit dichroism $\xi = 0.15$, corresponding to complete alignment of repressor molecules. Measurements of the dichroism at 248.2 nm revealed an opposite amplitude leading to a limit dichroism of −0.05, whereas the dipole moment $\mu_p = 3.5 \times 10^{-27}$ C m corresponds to that evaluated from the data measured at 280.4 nm. Addition of an equivalent quantity of the inducer tetracycline leads to a slight increase of the permanent dipole moment ($\mu_p = 3.9 \times 10^{-27}$ C m) and also to an increase of the absolute values found for the limit dichroism (0.16 at 280.4 nm and − 0.09 at 248.2 nm). Since tetracycline shows a maximum of absorbance at 365 nm, the dichroism of the protein–tetracycline complex could also be characterized at this wavelength: the limit dichroism is −0.45, and the dipole moment (3.7×10^{-27} C m) is consistent with the values given above. The dichroism of free tetracycline is negligible. Due to the high affinity of tetracycline for Tet repressor (Takahashi et al., 1986), the inducer should be associated quantitatively with the protein under the conditions of our experiments.

As shown by the example of DNA double helices [cf. Diekmann et al. (1982)], the existence of permanent dipole moments may be simulated by a saturation of a polarizability. However, in this case, the electrical parameters proved to be strongly dependent on the salt concentration. For comparison, the dichroism of Tet repressor has been measured at elevated salt concentration in the buffer T–Na. Again, the experimental data could only be fitted by a permanent dipole mechanism; the resulting dipole moment (3.9×10^{-27} C m) is equivalent to that obtained at low salt within experimental accuracy. Thus, the protein appears to be associated with a true permanent dipole moment.

Other useful information accessible from dichroism experiments is the rotational diffusion time constant, which can be evaluated from the dichroism decay. The rotation time constant obtained for the free repressor is 40 ns. Addition of tetracycline induces a slight decrease of the rotation time constant to 38 ns. However, due to a minimal sample time of 5 ns of the 7612D transient recorder and potential jitter phenomena, the accuracy of the rotation time constants is limited to about ±5 ns.

The experimental rotation time constant may be compared to the value expected for the limit case of a spherical shape of the protein. Using a molecular weight for the dimer of 47 000, a specific volume 0.74 cm^3/g calculated according to Zamyatnin (1972) from the amino acid content, and a standard hydration of 0.34 g of H_2O/g of protein, we arrive at a total volume of 8.43×10^4 Å^3. A sphere of equivalent volume has a radius of 27.2 Å and a rotation time constant of 20.9 ns. By comparison with the experimental time constant, we may conclude that the protein must be elongated. From the ratio of the rotation time constants, which are observed for the protein and calculated for the equivalent sphere, we may estimate dimensions of a prolate elipsoid (Cantor & Schimmel 1980), which is compatible with the experimental data. According to this estimate, the long axis of a prolate representing the protein dimer is 100 Å and the short axis is 40 Å.

Rotation Time Constants and Dichroism of Operator DNA and Operator–Repressor Complexes. As usual, the rotational

Table I: Rotational Time Constants of Operator DNA Fragments in the Absence (−) and Presence (+) of an Equivalent of Tet Repressor Protein, Which Is Sufficient To Saturate the Operator Sites[a]

		T-Mg		T-Mg-Na			
DNA	bp	−	+	−	+	+[b]	+[c]
RA1	73	438	480	438	477		
Tn10	76	447	516	452	509	514	492
pSC101	80	513	525	524	515	557	510
RP1	81	534	595	547	587	629	562
120	120	1480	1500	1535			
185	185	3390					

[a] All values, given in nanoseconds, are extrapolated to zero field strength. The numbers given for the fragments with 120 and 185 bp refer to the second, slow exponential. All data are measured at 20 °C. The estimated accuracy is ±2% for the fragments with chain lengths below 100 bp and ±4% for the other fragments. [b] Data obtained for complexes mixed at high salt and subsequently dialyzed to the buffer T-Mg-Na. [c] Data obtained for protein-DNA complexes after addition of tetracycline at a 1/1 stoichiometry with respect to protein monomers.

Table II: Limit Linear Dichroism (ξ_∞) at 248.2 nm for Operator-DNA Fragments and Tet Repressor-Operator Complexes[a]

		T-Mg		T-Mg-Na			
DNA	bp	−	+	−	+	+[b]	+[c]
RA1	73	−0.96	−0.95	−0.91	−0.90		
Tn10	76	−0.93	−0.90	−0.93	−0.83	−0.84	−0.83
pSC101	80	−0.99	−1.00	−0.97	−0.96	−0.84	−0.87
RP1	81	−0.96	−0.95	−0.92	−0.83	−0.86	−0.87
120	120	−0.81	−0.83	−0.86	−0.70		
185	185	−1.04					

[a] The notations are equivalent to those used in Table I. The ξ_∞ values were determined by least-squares fitting to the saturating induced dipole model (Diekmann et al., 1982) in the version of the "square root approximation" (Diekmann et al., 1984). [b] Same as footnote *b* of Table I. [c] Same as footnote *c* of Table I.

relaxation time constants have been evaluated from the dichroism decay curves. Due to stretching effects induced by the electric field (Porschke et al., 1984a; Porschke, 1986), the dichroism decay time constants show some dependence on the electric field strength, although the decay curves are measured in the absence of an electric field. The influence of these effects is considered by measurements with pulses of different field strength and extrapolation of the observed dependence to zero field strength by linear regression. The results are compiled in Table I. All the operator fragments used in this investigation were prepared by *Eco*RI restriction nuclease and thus had four single-stranded residues at each end. The influence of these residues on the rotational diffusion of the DNA fragments was evaluated by a comparison with blunt-ended fragments. For this purpose, rotational time constants of five *Hae*III fragments with 43, 69, 84, 179, and 256 bp were determined in the buffer T-Mg and fitted according to a weakly bending rod model in the form given by Hearst (1963). The persistence length of 340 Å and the "Stokes" diameter of 26.9 Å evaluated by this procedure can be used to calculate theoretical rotation time constants for any DNA fragment with chain lengths in the range from (at least) 43 to 256 bp. Comparison of the time constants measured for the *Eco*RI fragments with the theoretical values leads to effective hydrodynamic chain lengths for the *Eco*RI fragments. According to this evaluation, the length increment contributed by the four single-stranded residues at both ends is not constant for the various fragments. However, it is not very likely that this effect is due to the single-stranded ends. For example, the rotational time constant found for the fragment with 120 bp is relatively large, which is probably due to some contamination and/or aggregation. Another deviation is found for the fragment Tn10 with 76 bp, which exhibits a relatively low rotational time constant. It is tempting to speculate about potential curvature of this fragment. However, the fragment with 185 bp bears the same operator sequence, and yet its rotation time constant is consistent with expectation, within the limits of accuracy. In summary, some deviations are observed, the nature of which remains to be established. The experimental data obtained for the *Eco*RI fragments with chain lengths below 100 bp indicate that the four single-stranded residues at each end contribute an average length increment corresponding to approximately 5 bp.

Addition of protein equivalents sufficient for saturation of the operator sites leads to an increase of the rotation time constants (cf. Table I). These data may first be analyzed by the simple rule of thumb, according to which the time constant observed for the complex is the sum of the time constants of its components, provided that there is no major change of their hydrodynamic shape. The rule appears to be confirmed in several cases, suggesting the absence of major conformation changes upon complex formation. Deviations as observed for the example of pSC101 may be explained by incomplete binding, which was deduced previously from gel retardation experiments (Klock et al., 1985).

In some cases (cf. Tables I and II), the components have been mixed at high salt (T-Mg buffer + 0.1 M NaCl) to avoid irreversible formation of unspecific complexes, before the mixture was dialyzed to the low-salt buffer required for electrooptical measurements. This procedure did not affect the time constant observed for the complex formed with Tn10, whereas some increase of the time constants was found for the complexes formed with pSC101 and RP1. However, the changes remain relatively small and thus will not be discussed any further.

Addition of tetracycline to some complexes induces a reduction of the rotation time constant, as should be expected for tetracycline-induced dissociation of the protein from the DNA. In the case of Tn10, the rotation time constant observed in the presence of tetracycline indicates that part of the protein remains bound to the DNA, apparently in some unspecific complex.

Dichroism amplitudes and time constants have also been measured for a DNA fragment with 185 bp, which comprises the Tn10 tandem operator, in the absence and the presence of the repressor protein. The data for the protein complex with the 185 bp fragment have been collected in buffers T and T-Na and thus are not included in Tables I and II. The slow

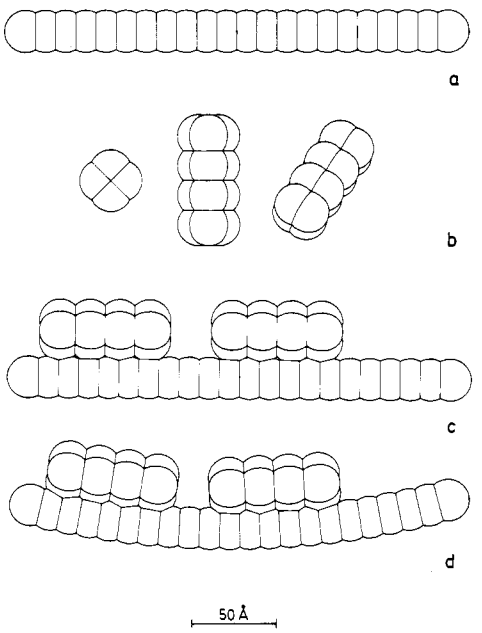

FIGURE 2: Bead models assembled by beads of radius 12.5 Å. (a) Tn10 DNA fragment represented by 22 overlapping beads. (b) Tet repressor represented by a "cylinder" from 16 overlapping beads in 3 different views. (c) Complex of two cylinder repressor molecules with straight Tn10 DNA. (d) Same as (c), but DNA smoothly bent to a radius of curvature 500 Å.

rotation time constant (τ_2) of the 185 bp fragment in buffer T remains almost unaffected (3.8 μs) upon addition of up to 4 equiv of protein dimers. Saturation of the operator sites in the buffer T–Na also did not induce any change of the slow rotation time constant (3.5 μs) within experimental accuracy. These results confirm our conclusion derived from measurements with the shorter DNA fragments that binding of the Tet repressor to its operator site does not induce any major change of the hydrodynamic dimensions of the DNA double helix.

The dichroism amplitudes (ξ) observed as a function of the electric field strength have been evaluated according to the saturating induced dipole model (Diekmann et al., 1982) in the form of the "square root approximation" (Diekmann et al., 1984). The limit values of the linear dichroism (ξ_∞) compiled in Table II also support the conclusion that repressor binding does not lead to any major changes of the DNA structure.

Simulation of Repressor–Operator Complexes by Bead Models. Experimental data on the rotational motion of DNA double helices and of proteins can be described reasonably well by, e.g., the weakly bending rod model and elipsoids of revolution, respectively, provided that these molecules are analyzed separately. These simple models cannot be used to describe protein–nucleic acid complexes, because their shape cannot be represented adequately by any of the standard geometric bodies. The analysis of complex structures from rotational time constants requires application of special techniques for the simulation of rotational diffusion by bead models. These techniques have been described in detail by Garcia de la Torre and Bloomfield (1981), who also provided substantial contributions to their development. A short description of the simulation procedure applied in the present investigation is given by Antosiewicz and Porschke (1988).

The first task is selection of a reasonable arrangement of beads, which reflects the known properties of the Tet repressor protein. According to the results described above, the Tet repressor is elongated, and its rotational relaxation can be described by a prolate elipsoid with a long axis of 100 Å and a short axis of 40 Å. Another object, which fulfills the "boundary conditions" of the total molecular volume (84 300 Å^3) and the experimental rotation time constant (40 ns) (cf. above), is a cylinder constructed from 16 beads of radius 12.5 Å as shown in Figure 2b. The length of the cylinder is 78 Å and its diameter 37 Å.

Table III: Modeling of Rotational Relaxation Time Constants (ns) for Operator Fragments by Linear Strings of Overlapping Beads[a]

DNA	exptl relaxation time (ns)	calcd relaxation times (ns)		
		r = 12.4 Å	r = 12.5 Å	r = 12.6 Å
RP1	529	541	543	546
Tn10	449	452	454	457
pSC101	519	510	512	515
RA1	442	425	427	429
$\sum(\tau_i^{\text{exptl}} - \tau_i^{\text{calcd}})^2$		523	495	538

[a] Rotational time constants are calculated as described by Antosiewicz and Porschke (1987) for various values of the bead radius (r). A reasonable representation of the experimental data is found for a bead radius of 12.5 Å, as judged from a minimum in the sum of squared deviations.

As described previously (Antosiewicz & Porschke, 1988), the rotational relaxation of DNA double helices has been simulated by a linear string of overlapping beads with initial dimensions as expected according to the general structure of B DNA and subsequent adjustment of bead diameters to the measured dichroism decay time constants. The four single-stranded residues at each end of the fragments were considered by a total length increment of 5 bp (cf. above). Since the time constants observed for the operator fragments do not follow exactly the expected function of their contour length, we have some difficulty in deriving a consistent set of bead parameters. A minimal deviation from the set of time constants observed for the fragments RP1, Tn10, pSC101, and RA1 is found for a bead radius of 12.5 Å (cf. Table III).

The complexes between the DNA fragments and the repressor are simply formed by alignment of the DNA and the protein beads into close contact, with the center of the protein at the center of the palindrom. We assumed a maximal overlap between protein and DNA beads corresponding to 3 Å. Both operators are combined with one protein dimer each. Since the resulting complex is not symmetric, more than a single rotational relaxation time constant is computed. However, one of these time constants is associated with almost all of the dichroism amplitude, and the other components do not contribute more than about 1%. We have checked whether a permanent dipole component resulting from attachment of the Tet repressor [cf. Crothers et al. (1978)] induces a change of the relative amplitudes of the various relaxation processes. Model calculations with several orientations of a permanent dipole showed that the slow process remained dominant in all cases of practical interest. It may be suspected that turning one of the proteins around the DNA with respect to the other one may affect the calculated rotation time constant seriously. However, alignment of the proteins at opposite sides of the DNA rather than at the same side leads to an increase of the rotation time constants by not more than about 5 ns.

For a simple comparison of calculated and experimental results, the calculated dichroism decay curves were fitted by single exponentials using a least-squares fitting procedure. As expected, the average time constant ($\bar{\tau}$) obtained by this procedure is very close to that of the process with the dominant amplitude. The $\bar{\tau}$ values (for straight DNAs in their complexes with repressor) are somewhat larger than the corresponding

Table IV: Rotational Time Constants (ns) Calculated for Tet Repressor–Operator Complexes According to Bead Models[a]

	calcd dichroism decay						
	straight DNA		500-Å bent DNA		av $\bar{\tau}$ from least-squares fit (ns)		exptl relaxation time (ns)
DNA	rel amplitude	relaxation time (ns)	rel amplitude	relaxation time (ns)	straight DNA	500-Å bent DNA	
RP1	$0.997E + 0$	627	$0.998E + 0$	598	625	596	595
	$0.272E - 2$	352	$0.190E - 2$	330			
	$0.522E - 5$	153	$0.551E - 3$	142			
Tn10	$0.993E + 0$	543	$0.995E + 0$	517	540	515	516
	$0.651E - 2$	322	$0.502E - 2$	303			
	$0.424E - 5$	146	$0.398E - 3$	137			
pSC101	$0.999E + 0$	587			586		525
	$0.116E - 2$	339					
	$0.536E - 5$	151					
RA1	$0.999E + 0$	500	$0.999E + 0$	479	499	478	480
	$0.670E - 3$	309	$0.467E - 3$	290			
	$0.100E - 4$	145	$0.323E - 3$	134			

[a] For details, see text.

experimental values—in particular in the case of the fragment pSC101 (cf. Table IV). Gel retardation experiments demonstrated that binding of the Tet repressor to fragment pSC101 is relatively weak (Klock et al., 1985), and thus most of this fragment apparently remained free during our dichroism measurements. The deviations observed for the other fragments, where binding of the repressor should be quantitative, may be attributed to some bending of the DNA. Assuming smooth bending of DNA, we get excellent agreement of simulated and experimental time constants for a radius of curvature 500 Å (cf. Table IV). As shown in Figure 2, this radius of curvature corresponds to a relatively small degree of bending. Since the accuracy of both experimental and simulated time constants is limited to approximately ±10 ns, we cannot completely rule out that the DNA remains straight. Conversely, we estimate that the maximum degree of bending consistent with the experimental data corresponds to a radius of curvature ~400 Å.

Discussion

The analysis of the Tet system by electrooptical procedures proved to be particularly useful, because all the components and their complexes could be characterized by the same approach. Even the anisotropy of the protein turned out to be sufficiently large for an evaluation of the main parameters accessible by electrooptical measurements. Obviously, the protein dimensions derived from the rotation time constant are model dependent. Nevertheless, it may be concluded that the protein dimer must be elongated like a prolate elipsoid with a long axis of 100 Å and a short axis of 40 Å. These dimensions may be compared with the number of approximately 25 bp of the operator DNA which are protected against cleavage by deoxyribonuclease I upon complexation with the Tet repressor (Hillen et al., 1984). Since 25 bp extend over ~85 Å, the long axis of the protein appears to be parallel to the DNA in the complex. The agreement of nuclease protection data and the rotational time constant is more favorable for a cylinder model of the protein formed from 16 overlapping beads of radius 12.5 Å each. The length of the cylinder (78 Å) indicates that again its long axis should be parallel to that of the DNA.

The dichroism amplitudes observed for the repressor as a function of the electric field strength demonstrate the existence of a permanent dipole moment. The magnitude of this dipole is surprisingly large (3.5×10^{-27} C m corresponds to 1050 D) and indicates a strongly asymmetric distribution of charges. If the structure of the Tet repressor is indeed similar to that observed for other helix–turn–helix protein dimers, the electrical asymmetry is an obvious consequence from its asymmetric structure. Apparently, the dipole contributes to the interaction of the protein with the DNA. The dipole should also be useful for the dynamics of complex formation, because the contact surface may be directed toward the operator site by dipole interactions and thus the influence of "steric" factors may be reduced. Recently, a large permanent dipole moment has also been identified for the *lac* repressor (Porschke, 1987). Thus, permanent dipole moments may well be characteristic of DNA binding proteins.

The rotation time constants evaluated for the DNA fragments do not follow exactly the dependence expected from their contour length. Unfortunately, the deviations cannot be interpreted quantitatively due to an uncertainty concerning the contribution of the four single-stranded residues at each end resulting from the preparation by *Eco*RI restriction nuclease. By comparison with a set of blunt-ended fragments, we find an average contribution of both single-stranded ends corresponding to a length increment of 5 bp. The deviation from this rule found for individual fragments is ±1 bp. Although the rotation time constant for a given solution of a DNA fragment with, e.g., 95 bp can be determined at an accuracy better than ±1 bp [cf. Porschke et al. (1982)], a comparison of different fragments may be misleading, unless contaminations can be excluded completely. It is known, for example, that addition of spermine in the micromolar concentration range may induce a substantial reduction of DNA rotation time constants (Porschke, 1986). For this reason, small differences of rotation time constants observed for different samples should be interpreted with caution. The samples used in our present investigation have been dialyzed extensively to remove contaminations of low molecular weight. Thus, deviations from a smooth dependence of the rotational time constants on the contour lengths should be "intrinsic" and reflect deviations from the standard B form of the double helix. However, these deviations are relatively small.

The main goal of our present investigation was the analysis of complexes between repressor and operator in solution. The comparison of experimental rotation time constants with calculations according to bead models indicates that the formation of repressor–operator complexes is not accompanied by any major change of the hydrodynamic dimensions of the components. Since the rotation time constant of the complex is dominated by the contribution of the DNA, we cannot exclude changes of the protein structure, whereas any major change of the DNA dimensions can be ruled out completely. Thus, bending of operator DNA by the Tet repressor is not nearly as large as observed previously for the case of promotor DNA induced by the cyclic AMP receptor protein in the presence of cAMP (Porschke et al., 1984a; Antosiewicz &

Porschke, 1988). This result is of general interest, partly because both proteins are members of the class of helix–turn–helix proteins. Apparently, the structure of protein–DNA complexes may be quite different, even if formed from proteins bearing the same type of DNA binding motif. One may speculate about possible relations between the structures and their function. It is tempting to relate the strong bending of DNA induced by the cyclic AMP receptor with its function as gene activator and the absence of strong bending in the case of the Tet protein with its function as repressor. For any general conclusions, however, the structure of more specific protein–DNA complexes should be analyzed. For this purpose, electrooptical procedures will be particularly useful because of their potential to analyze complex structure *in solution* at a remarkably high sensitivity.

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